

COMPARISON OF BIOCHEMICAL RESPONSES BETWEEN SINGLE AND REPEATED  
EXPOSURES TO AIR AT 6.7 ATA

by

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## ABSTRACT

U. S. Navy diver volunteers were subjected to short (45 minute) exposures to air at 6.7 ATA. The experimental protocol was designed to compare the recoveries from single exposures with those from similar dives employing 3 day inter-dive intervals. Serum and 24 hour urine samples were collected for 5 days prior to and for 7 and 10 days, respectively after the dives. Serum and urinary minerals, electrolytes, and protein metabolites as well as serum enzymes and urinary steroids were measured. The overall effects of air at 6.7 ATA on 19 serum parameters was very pronounced at one hour post-dive. Following a single dive, the response declined sharply to a low after 3 days. This was followed by a second response peak after 5 days and a subsequent gradual decline. Following the 3-day-repeat dives, a secondary peak occurred on the 3rd day following the second dive. The response to the diving stress as indicated by 14 urine parameters showed a small increase on the first two post-dive days followed by a decrease on the third. After a single dive, the consequences of the exposure continued to increase for up to 7-8 days. When a second dive was made on the 3rd day after the initial exposure, a very large response occurred on the 2nd day after the second dive, with a rebound on the 3rd day, and secondary peaks on the 4th and 6th day. These studies support previous observations that effects of hyperbaric exposure continue for several days following pressurization. Repeated exposures within the recovery period alter the pattern of recovery.

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# COMPARISON OF BIOCHEMICAL RESPONSES BETWEEN SINGLE AND REPEATED EXPOSURES TO AIR AT 6.7 ATA

## INTRODUCTION

Exposure to increased pressure and decompression from elevated pressures result in alterations in blood and urinary minerals, electrolytes, protein metabolites, and hormones<sup>1,13,15,16</sup>. Hyper-ventilation and fluid shifts<sup>17</sup>, increases in corticosteroid and/or aldosterone secretion<sup>2</sup>, or alterations in membrane permeability due to inert gas narcosis<sup>3</sup> have been implicated in these changes.

In addition, atypical metabolite levels have been observed in sera and urine for extended periods following decompression stress. Severely decompressed rats exhibit mineral and electrolyte alterations for up to 5 days post-decompression<sup>6</sup>. In men exposed to air at 2 and 7 ATA for 45 minutes, changes are evident in urinary minerals, electrolytes and protein metabolites for up to 4 days<sup>7</sup>. Similar results have been observed for up to 7 days following simulated dives in air to 100 FSW for 60 minutes<sup>18</sup>.

Since exposures to hyperbaric environments result in biochemical alterations of several days duration, the relationship between interdive interval and the cumulative effect of repeated pressurizations was studied by Heyder and Tappan<sup>8</sup> using a 28-day interval between exposures to air at 6.7 ATA. Although no apparent biochemical carry-over was observed for this extended interval, certain potential effects of diving appear cumulative<sup>4,12</sup> and may be related to

the frequency and type of pressure experience. Therefore, the purpose of this series of experiments was to investigate the effect of re-exposure to pressure during the recovery period following hyperbaric insult.

## MATERIALS AND METHODS

Seven Navy divers participated in the studies to be reported. The simulated dives commenced at 0830, exposing one diver per day to 6.7 ATA (188 feet of sea water equivalent). The men were compressed at the rate of 75 ft/min. Bottom time was 40-45 minutes and decompression was performed according to the U. S. Navy Diving Manual using the air table for 50-minute exposure to 190 feet<sup>19</sup>. Three days after his initial dive each diver was again subjected to the same procedure. Twenty-eight days after participating in the above experiments, four of the divers who had not participated in the earlier 28 day-interval study<sup>8</sup> made single dives to 6.7 ATA.

Prior to his first dive each man collected five consecutive 24-hour acidified urine specimens to serve as controls. Since no individual voidings were collected during the dives, the urine sample for the first post-dive days included the urine produced during the dive periods.

Twenty-four-hour urine collections were made for 13 post-dive days (3 days after the initial and 10 days after the second dives). During these studies the men refrained from eating gelatin, ice-

From Table I it can be seen that following a single dive to 6.7 ATA using air, the greatest responses in the serum seem to occur 1 hour after surfacing. Phosphorus, lactic dehydrogenase (LDH), glutamic-oxalacetic transaminase (SGOT), albumin, globulin, and total protein were observed to increase while ionized calcium, calcium/phosphorus ratio, sodium, and urea nitrogen decrease. The ionized calcium decrease undoubtedly reflects binding to the elevated protein levels which may result from the fluid shifts to extracellular spaces that have been observed during hyperbaric exposures <sup>11</sup>. The increases in lactic dehydrogenase (LDH) and glutamic-oxalacetic transaminase (SGOT) observed at one hour post-dive suggest tissue damage and leakage of these enzymes into the circulation. The decrease in the calcium/phosphorus ratio at 1 hour and 1 day after the dive reflects the increase in serum phosphorus since total calcium level remains essentially unchanged.

The post-dive changes presented in Table II demonstrate a significant increase in phosphorus and a decrease in calcium/phosphorus ratio comparable to the effects following the first dive. The most striking feature observed in all these data, however, is the great number of significant changes that occur 1 hour after surfacing from the second dive, at a time when residual effects from the first dive presumably still exist. Significant decreases in calcium, calcium/phosphorus ratio, sodium, potassium, urea nitrogen concentration, and alkaline phosphatase and lactic dehydrogenase activity occur at this time.

A comparison between the effects on the serum parameters of the single dives (Table I) and repeat dives following a 3-day interval (Table II) is presented in Table III.

Comparisons are made for single dive control vs. repeat dive control, 1 hour post-dive vs. 1 hour post-dive, etc. Since the means and standard errors of the mean are presented in Tables I and II they are not included in Table III and only those results which differ significantly at the 5% level or better are indicated. The direction of the change for the multiple dives as compared to the single dive is indicated by the arrows. When considering the effects of exposure to 6.7 ATA in air on the 19 serum parameters measured, it is evident that the greatest responses occur one hour after return to the surface following a single exposure and to an even greater extent following a dive repeated after a three-day interval. Most of the other changes noted seem to reflect phase differences in the superimposed cyclic recovery curves so that maxima and minima of the two sets of data occasionally occur in proximity resulting in apparent differences. With respect to the changes in cholesterol levels, the number of paired comparisons was small (3), and we consider the results reported to be of questionable validity.

The importance of all these responses to the overall long term health of the divers remains obscure. Changes in lactic dehydrogenase (LDH) and glutamic-oxalacetic transaminase (SGOT) would suggest tissue damage while alterations in alkaline phosphatase, calcium, phosphorus, and calcium/phosphorus

ratio may indicate bone metabolism involvement. Sodium and potassium changes and the markedly elevated serum osmolality observed 1 hour after the second dive suggest that fluid shifts occur at this time. Fluid shifts resulting from hyperbaric exposures are well documented<sup>1,11</sup>.

### Urine

Table IV (Appendix) presents the combined data of all the total urinary excretion results obtained from the seven subjects making single dives to 6.7 ATA while Table V (Appendix) presents those total excretion data acquired during the study of repetitive dives.

From Table IV it can be seen that a single dive to 6.7 ATA results in latent excretory changes which become significant especially for ketosteroids, by the third post-dive day and remain depressed for the remainder of the collection period. Similar reductions in 17-ketosteroid excretion following brief hyperbaric exposures have been reported previously<sup>7,8</sup>. Since ketosteroid excretion is an indicator of adrenocortical stress<sup>5,14</sup> it is apparent that full recovery from short-term stress requires from several days to at least a week. One other significant change in excreted substances occurs late in the recovery period; calcium during the 7-8th post-dive days. This delayed mineral loss requires further observation with regard to its potential relationship to necrotic bone deterioration in divers.

The post-dive urinary changes shown in Table V present a considerably different picture. When dives are repeated after a 3-day interval, the greatest changes occur on the 2nd day following the second dive. Sodium, potassium, uric acid, osmoles, and ketosteroids all increase significantly from their control values. These increases in excretion products result, at least in part, from the increases in fluid intake and urine produced. On the 4th post-dive day an increase occurs in urine volume and the total osmoles excreted. This increase in urine output may account for the significantly increased fluid intake observed on the 5th day.

As with the serum parameters of Table III, a comparison of interdive effects on urinary responses is presented in Table VI. Again only those results which differ significantly at the 5% level or better are indicated. It may be noted that significant interdive changes in creatinine, osmolality, and urea nitrogen occur following the second dive in the series in the complete absence of post-dive changes following the first dive. After the second day only potassium changes were noted.

Integration of all of the data for interpretation and evaluation of the changes in the parameters measured in both serum and urine is difficult at this stage of our understanding of diving stresses. The kind and/or direction of the changes often seem to be related to individual diver variability or subtle differences in diving procedures such as slight variations in compression rate, or amount of work performed. One

approach which we have explored for evaluating the total biochemical response to hyperbaric stress uses the sum of the absolute size of the fractional changes from control values, of all parameters measured for each period. A second approach utilizes a summation of all the t-values of the paired analyses of experimental vs. control data in the same manner. Although the values from the two summations tend to be highly correlated the sum of the t-values has an advantage in that it is less influenced by erratic data and seems promising for future evaluation of stress responses. To illustrate these points, Tables VII and VIII present the sum of the t-values and the sum of the relative mean differences for serum and urine respectively for the single and the 3 day repeat dives. It should be pointed out that although absolute numbers are presented in these tables they do not represent absolute indices of stress. For example, we cannot say that the 2nd post-dive day following the second dive is 3.5 times as stressful as the 1st day (Table VIII). For ease of comparison of hyperbaric effects, Figures 1 and 2 present graphically only the sum of the t-value data.

Figure 1 demonstrates the very pronounced overall parametric responses that occur 1 hour after a dive. Following single dives, the sharp decline to a low after 3 days, with a subsequent second peak of activity after 5 days, and a decrease again by 7 days presents a multiphasic pattern similar to that observed by Jacey, et al<sup>10</sup> in the hematological parameters in animals subjected to hyperbaric stress. Of particular

interest, however, is the apparent telescoping or decrease in cyclic period length of the biochemical responses in the serum after a second dive to 6.7 ATA. The secondary peak, which occurred 5 days after the single dive, is seen to occur in the repetitive protocol on the 3rd day following the second dive.

The total response of urinary excretory products following the hyperbaric exposures is depicted in Figure 2. In this figure the summed t-data are shown for each day of the post-dive collection period; whereas in the interest of conciseness, the tables of excretion data have presented results from combined days. Figure 2 illustrates the seeming oscillatory nature of post-dive urinary excretory patterns.

After the initial pressurization, there is a small increase in response for the first two post-dive days which is followed by a slight decrease on the third day. When only one dive is performed, urinary changes then gradually increase and reach peaks on the 6th and 8th post-dive days followed by a sharp decline on the 10th day. This is not unlike the pattern of excretion observed following 1-hour exposure to simulated dives of 100 FSW<sup>18</sup>. However, when a second dive is performed on the third day after the initial hyperbaric exposure, a different response pattern emerges. A very large increase in response occurs on the 2nd day after the second dive. This is then followed by a profound rebound effect on the 3rd day, with secondary peaks on the 4th and 6th days, and a gradual dampening of oscillations thereafter.

These studies further support the previous observations that the total

TABLE VIII

SUM OF PAIRED *t*-VALUES AND RELATIVE MEAN DIFFERENCES FOR 14 URINE PARAMETERS FOLLOWING EXPOSURES TO AIR AT 6.7 ATA

<u>POST-DIVE PERIOD</u>	<u>SINGLE DIVE</u>		<u>POST-DIVE PERIOD</u>	<u>3-DAY INTERVAL REPEAT DIVE</u>	
	<u><math>\Sigma RD</math></u>	<u><math>\Sigma t</math></u>		<u><math>\Sigma RD</math></u>	<u><math>\Sigma t</math></u>
1 Day	1.155	10.706	1 Day	3.619	10.760
2 Days	1.407	11.836	2 Days	1.454	10.885
3 Days	1.089	9.964	3 Days	1.203	7.214
4 Days	1.293	11.420	1 Day	1.992	9.087
5-6 Days	1.159	13.404	2 Days	7.108	32.084
7-8 Days	1.725	21.372	3 Days	1.003	4.425
9-10 Days	1.093	10.999	4 Days	3.068	18.650
			5-6 Days	2.561	15.381
			7-8 Days	2.016	11.658
			9-10 Days	1.688	12.516

biochemical effects of hyperbaric exposure continue for many days following pressurization. Moreover, they reveal that a repeat exposure during the recovery period stimulates additional responses and alters the pattern of the recovery. How these patterns are further altered by several additional dives and by the timing of such exposures, as well as the significance of these changes will be of continuing interest.

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APPENDIX

TABLES I, II, IV, V

TABLE I (continued)

EFFECT OF A SINGLE EXPOSURE TO AIR AT 6.7 ATA ON SERUM MINERALS,  
 ELECTROLYTES, AND PROTEIN METABOLITES. MEAN  $\pm$  SEM.  
 Asterisk (\*) indicates  $p \leq .05$  by paired t-test.

PERIOD	#	LDH	SGOT	GLUC	CHOL	BILI	TP	ALB	GLOB	A/G	ALKP
CONTROL		137.6 4.6	25.29 1.81	99.5 3.7	197.5 13.4	0.746 0.144	7.13 0.16	4.39 0.08	2.74 0.08	1.61 0.04	48.17 6.98
1 HR		150.0 6.4	27.14 1.81	106.5 4.9	204.1 11.7	0.875 0.206	7.49 0.19	4.61 0.11	2.87 0.09	1.61 0.04	48.37 6.28
	*	*	*	*			*	*	*	*	
1 DY		133.6 5.6	22.75 1.75	105.2 6.1	194.9 11.6	0.688 0.117	7.06 0.20	4.28 0.14	2.80 0.07	1.53 0.04	49.62 6.43
	*									*	
3 DY		137.0 7.7	23.62 2.01	98.2 5.8	191.7 11.8	0.612 0.116	7.06 0.08	4.31 0.05	2.75 0.08	1.58 0.05	47.62 6.21
5 DY		133.5 7.9	23.25 4.33	90.7 4.0	184.7 15.8	0.575 0.048	6.90 0.17	4.17 0.07	2.73 0.14	1.54 0.08	50.25 14.79
7 DY		130.0 10.2	25.25 4.82	106.5 3.1	187.5 13.2	0.575 0.180	6.95 0.30	4.25 0.20	2.70 0.13	1.58 0.06	47.50 9.39
						*					

#LDH = lactic dehydrogenase, mU/ml  
 SGOT = glutamic-oxaloacetic  
       transaminase, mU/ml  
 GLUC = glucose, mg%  
 CHOL = cholesterol, mg%  
 BILI = bilirubin, mg%

TP = total protein, g%  
 ALB = albumin, g%  
 GLOB = globulin, g%  
 A/G = albumin globulin ratio  
 ALKP = alkaline phosphatase, mU/ml

TABLE II (continued)

EFFECT OF TWO EXPOSURES TO 6.7 ATA PERFORMED 3 DAYS APART ON SERUM  
MINERALS, ELECTROLYTES, AND PROTEIN METABOLITS. MEAN  $\pm$  SEM.  
Asterisk (\*) indicates  $p \leq .05$  by paired t-test.

PERIOD	#	LDH	SGOT	GLUC	CHOL	BILI	TP	ALB	GLOB	A/G	ALKP
CONTROL		155.2 11.4	27.67 2.07	102.2 3.1	211.9 18.4	0.652 0.122	7.13 0.16	4.32 0.13	2.81 0.09	1.55 0.06	51.24 6.59
1 HR		156.3 8.4	28.14 2.06	118.9 8.7	216.3 15.6	0.657 0.092	7.43 0.18	4.56 0.13	2.87 0.09	1.60 0.06	52.14 6.70
1 DY		146.6 8.9	25.57 2.55	106.0 4.4	212.9 13.4	0.629 0.115	7.04 0.20	4.23 0.14	2.80 0.09	1.51 0.04	50.43 6.30
3(0) DY		150.6 12.7	28.00 3.12	96.7 4.0	212.0 15.8	0.614 0.137	7.14 0.07	4.33 0.07	2.81 0.07	1.55 0.05	47.86 5.87
1 HR		138.8 13.2	26.83 3.56	110.7 8.9	199.3 16.2	0.717 0.202	6.97 0.16	4.23 0.09	2.73 0.12	1.56 0.07	43.67 6.24
1 DY		152.0 11.9	28.71 3.81	96.0 7.4	206.9 15.4	0.600 0.120	7.06 0.13	4.26 0.09	2.80 0.07	1.53 0.05	50.86 7.62
3 DY		159.7 10.0	27.86 2.71	97.9 5.0	207.4 19.1	0.557 0.111	6.93 0.15	4.24 0.13	2.69 0.08	1.59 0.06	51.86 7.72
5 DY		144.0 9.4	24.43 1.74	104.7 4.6	203.6 13.3	0.600 0.113	7.06 0.09	4.38 0.08	2.76 0.07	1.57 0.06	49.57 7.91
7 DY		156.0 7.9	28.29 3.84	100.1 5.3	214.4 20.5	0.614 0.076	7.27 0.12	4.41 0.12	2.86 0.07	1.55 0.06	49.57 6.87

#LDH = lactic dehydrogenase, mU/ml

SGOT = glutamic-oxaloacetic  
transaminase, mU/ml

GLUC = glucose, mg%

CHOL = cholesterol, mg%

BILI = bilirubin, mg%

TP = total protein, g%

ALB = albumin, g%

GLOB = globulin, g%

A/G = albumin globulin ratio

ALKP = alkaline phosphatase, mU/ml

TABLE IV (continued)

FLUID INTAKE AND TOTAL 24 HOUR URINARY EXCRETION FOLLOWING A SINGLE  
EXPOSURE TO AIR AT 6.7 ATA. MEAN  $\pm$  SEM.  
Asterisk (\*) indicates  $p \leq .05$  by paired t-test.

PERIOD	#NA/K	UN	UA	CRT	OSM	KS
CONTROL	3.89 0.41	7.69 0.73	0.822 0.103	2.25 0.31	0.871 0.061	19.28 1.52
1 DY	4.28 0.60	6.91 0.87	0.796 0.087	1.96 0.25	0.824 0.084	17.60 2.15
2 DY	3.09 0.41	6.79 0.75	0.719 0.094	2.03 0.29	0.821 0.063	15.93 2.36
3 DY	3.66 0.47	7.69 0.94	0.720 0.088	1.88 0.19	0.860 0.083	15.06 1.92 *
4 DY	3.93 0.60	8.58 0.83	0.952 0.083	2.18 0.23	0.990 0.064	16.62 1.40 *
5-6 DY	3.49 0.25	8.54 0.80	0.791 0.094	2.02 0.19	0.968 0.060	15.92 1.56 *
7-8 DY	2.89 0.34	7.13 0.97	0.810 0.082	1.85 0.23	0.851 0.068	14.58 1.92 *
9-10 DY	3.71 0.37	7.83 1.05	0.886 0.111	1.99 0.25	0.971 0.086	14.04 2.26 *

#NA/K = sodium potassium ratio  
UN = urea nitrogen, g  
UA = uric acid, g

CRT = creatinine, g  
OSM = osmoles  
KS = ketosteroids, mg

TABLE V (continued)

FLUID AND TOTAL 24 HOUR URINARY EXCRETION FOLLOWING TWO EXPOSURES TO  
AIR AT 6.7 ATA PERFORMED 3 DAYS APART. MEAN  $\pm$  SEM.  
Asterisk (\*) indicates  $p \leq .05$  by paired t-test.

PERIOD	#NA/K	UN	UA	CRT	OSM	KS
CONTROL	3.38 9.78	8.85 1.56	0.554 0.107	1.97 0.22	0.750 0.118	16.75 2.98
1 DY	3.98 1.81	8.64 0.97	0.589 0.105	2.14 0.26	0.764 0.085	19.06 3.35
2 DY	3.20 0.77	7.94 1.18	0.546 0.138	2.57 0.60	0.760 0.125	18.87 3.26
3(0) DY	2.97 0.71	8.06 1.67	0.411 0.110	1.99 0.41	0.787 0.127	17.68 3.75
1 DY	3.49 0.59	7.89 1.02	0.817 0.277	2.55 0.33	0.789 0.066	18.23 2.96
2 DY	5.02 1.81	10.84 1.06	1.283 0.370	2.96 0.53	1.609 0.895	23.86 4.15
3 DY	3.85 0.74	8.88 0.94	0.538 0.122	1.77 0.12	0.771 0.059	17.45 3.88
4 DY	4.05 0.88	9.48 1.26	0.923 0.272	2.26 0.21	0.913 0.114	26.57 3.28
5-6 DY	3.54 0.54	10.17 1.17	0.958 0.319	2.61 0.24	0.875 0.083	21.84 3.73
7-8 DY	3.71 0.59	10.27 1.23	0.705 0.125	2.21 0.33	0.859 0.081	19.36 2.97
9-10 DY	3.84 0.98	9.50 0.84	0.731 0.223	1.88 0.15	0.858 0.084	26.17 2.97

#NA/K = sodium potassium ratio  
UN = urea nitrogen, g  
UA = uric acid, g

CRT = creatinine, g  
OSM = osmoles  
KS = ketosteroids, mg

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